IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Morris, et al. Art Unit : 1642
Serial No. : 09/847,356 Examiner : Alana M. Harris
Filed : May 3, 2001
Title : REOVIRUS CLEARANCE OF RAS-MEDIATED NEOPLASTIC CELLS FROM MIXED CELLULAR COMPOSITIONS

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Commissioner for Patents
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DECLARATION OF MATTHEW C. COFFEY, PH.D. UNDER 37 C.F.R. § 1.132

1. I am a co-inventor of the above-referenced application. I am also Vice President and Chief Scientific Officer of Oncolytic Biotech Inc., assignee of this application.

2. I received a Ph.D. from University of Calgary in 1998. I have extensive experience in the areas of virology and oncolytic viral therapy. A copy of my *curriculum vitae* is attached herewith as Exhibit A.

3. I am familiar with and understand the specification and current claims, which are drawn to the use of reovirus to remove ras-mediated neoplastic cells from cellular compositions, including those comprising hematopoietic stem cells harvested from blood.

4. I understand that there is an Office Action outstanding in this application. The Office Action was mailed on September 22, 2004, rejecting this application on the ground of obviousness over various combinations of the following references:

- Gulati (J. Hematotherapy 2:467-471, 1993);
- Coffey et al. (Science 282:1332-1334, 1998);
- Freshney (Culture of Animal Cells: A Manual of Basic Technique, second edition, New York, NY 1987);

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- U.S. Patent No. 6,136,307;
- U.S. Patent No. 5,861,159;
- Nordon et al. (Artificial Organs 20(5):396-402, 1996).

5. In my opinion, the current claims are not obvious in view of the cited references because a skilled artisan, at the time this application was filed, would have recognized that hematopoietic stem cells harvested from blood have an activated ras pathway. Since cells with an activated ras pathway are prone to reovirus infection, the skilled artisan would not have been motivated to treat such hematopoietic stem cell compositions with reovirus. This notion is discussed in further detail below.

6. Prior to the mid 1980s, it was a standard procedure to collect hematopoietic stem cells from bone marrow for the treatment of various malignancies. It was subsequently discovered that there existed peripheral blood progenitor cells (PBPC). This new modality gradually replaced bone marrow as a source of stem cells. As pointed out in Winter[1], which is incorporated by reference in the present application and submitted with the IDS filed July 31, 2001, PBPCs had virtually replaced the traditional bone marrow autologous graft throughout the world (page 1642, right column, first paragraph under "Stem-Cell Source" of Exhibit B). PBPC collection has a number of advantages over collection from bone marrow, including: 1) it is generally less painful and does not require general anesthesia; 2) it is less expensive and; 3) it results in faster hematological recovery.

7. A key concept in the use of PBPC transplantation is the fact that hematopoietic cytokines can mobilize a large number of progenitor cells into the circulation (reviewed in Reddy[2]). Granulocyte colony stimulating factor (G-CSF) and granulocyte macrophage colony stimulating factor (GM-CSF) are the most commonly used factors for immobilization, and G-CSF was found to be more effective than GM-CSF (page 64, right column, first paragraph under "Hematopoietic cytokines" of Exhibit C). For example, allogeneic transplant donors are generally mobilized with daily subcutaneous injections of 10μg/kg of G-CSF for 5 days (abstract of Exhibit C).

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[1] Winter, "High-dose therapy with stem-cell transplantation in the malignant lymphomas", Oncology 13(12):1635-1645 (1999). Attached herewith as Exhibit B.

[2] Reddy, "Mobilization and collection of peripheral blood progenitor cells for transplantation", Transfusion and Apheresis Science 32:63-72 (2005). Attached herewith as Exhibit C.

8. It has been known in the art that both G-CSF and GM-CSF stimulate proliferation of hematopoietic cells by activating the ras pathway (see, e.g., Rausch and Marshall[3]; Bashey et al.[4]; Satoh et al.[5]). Since PBPCs are typically collected using G-CSF and/or GM-CSF, a skilled artisan would expect PBPCs to have an activated ras pathway and be prone to reovirus infection. Consequently, the skilled artisan would not have been motivated to treat PBPCs with reovirus.

9. However, the present invention demonstrates that when PBPCs were mixed with various ras-mediated neoplastic cells (MCF7, SKBR3 or MDA MB 468 cells), reovirus treatment resulted in selective killing of the neoplastic cells rather than hematopoietic stem cells (Example 1 of the present application (page 20)). Moreover, reovirus treatment neither inhibited cell proliferation nor altered differentiation potential of hematopoietic stem cells (Example 3 of the present application (pages 21-22)). It is surprising that these highly proliferative (through ras activation/signaling), undifferentiated cells do not support reovirus replication following their stimulation with G-CSF. It is particular surprising since reovirus replicates in other highly proliferative, undifferentiated cells such as the cells of the Peyers patch – the natural site of reovirus replication in the human host – the reason for this is currently not understood. Thus, the present invention demonstrates that reovirus can be successfully used to selectively remove ras-mediated neoplastic cells from a cellular composition comprising hematopoietic cells, which was not obvious to skilled artisans at the time this application was filed.

10. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by find or imprisonment, or both, under Section 1001 of Title 18 of the United States

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[3] Rausch and Marshall, "Cooperation of p38 and extracellular signal-regulated kinase mitogen-activated protein kinase pathways during granulocyte colony-stimulating factor-induced hemopoietic cell proliferation", J Biol Chem. 274(7):4096-105 (1999). Attached herewith as Exhibit D.

[4] Bashey et al., "Proliferative but not nonproliferative responses to granulocyte colony-stimulating factor are associated with rapid activation of the p21ras/MAP kinase signalling pathway", Blood 83(4):949-57 (1994). Attached herewith as Exhibit E.

[5] Satoh et al., "Involvement of ras p21 protein in signal-transduction pathways from interleukin 2, interleukin 3, and granulocyte/macrophage colony-stimulating factor, but not from interleukin 4", Proc Natl Acad Sci U S A. 88(8):3314-8 (1991). Attached herewith as Exhibit F.

Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Respectfully submitted,

Date: March 21 05

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